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REVIEW

Cell-mediated Immunity to Influenza Virus Infections: From the Perspective to the Vaccine Development against Highly Pathogenic Avian Influenza

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Abstract

Inactivated vaccines have been incorporated in the control strategies for highly pathogenic avian influenza (HPAI) in several countries. However, these conventional vaccines confer protective immunity in the hemagglutinin (HA) subtype-specific manner and inevitably promote antigenic drifts in HA of target viruses. Therefore, the efficacy of the conventional vaccines needs to be evaluated occasionally to assure that they still provide immunity against emerging or circulating field virus strains. To cover these pitfalls, novel vaccine strategies which target conserved viral antigens are currently being investigated. We hypothesize that such vaccines can be developed by utilizing cytotoxic $CD8^+$ T cell-mediated immunity. However, we do not fully understand the mechanisms of the generation, maintenance and recall of cell-mediated immunity to influenza virus infections. Here we briefly review the current knowledge of cell-mediated immunity to influenza virus infections based on the studies using mouse models and discuss the future application of this immunological arm to the vaccines against HPAI in poultry.

Discipline: Animal health
Additional key words: $CD8^+$ T cells, recall response, T-cell memory

Introduction

Highly pathogenic avian influenza (HPAI) is now widely spreading worldwide and causing great economic loss in the poultry industry. In addition, the number of direct transmissions of H5N1 HPAI viruses to humans is concurrently increasing. Recently, Mase and his colleagues clearly demonstrated that only one amino acid substitution can endow HPAI virus with high lethality in mammals[19]. These have raised serious concerns about the risk of a pandemic of HPAI viruses in humans. Therefore, strategies for the efficient control of HPAI are urgently needed[10,11].

Farm biosecurity and stamping-out of infected poultry flocks are primary strategies for the control of HPAI[2]. However, farm biosecurity is difficult to be applied to semi-industrial and backyard poultry farming. This partly contributes to the current endemic situation of HPAI in several developing countries. In addition, the preemptive killing of great numbers of poultry has raised a question on this strategy even in advanced countries from economic and ethical points of view.

Although oil-emulsified inactivated whole virus vaccines have been incorporated as an additional strategy in the control of HPAI in several countries[2], these conventional vaccines have several disadvantages. The inactivated vaccines confer protective immunity by eliciting systemic neutralizing antibodies against hemagglutinin (HA) on the virus surface[29]. This immunological mechanism inevitably promotes antigenic drifts in HA of target viruses. For example, Mexico has been using the vaccines since 1995 and controlling the emergence of highly pathogenic H5N2 virus. However, the vaccines have si-

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multaneously resulted in an endemic of low pathogenic H5N2 antigenic variants on which the vaccines are less effective[17]. Although several governments have been stockpiling inactivated vaccines based on H5N1 viruses that have been circulating over the last few years, the efficacy of these vaccines needs to be evaluated occasionally to assure that they still provide protective immunity against circulating or emerging field virus strains, especially in the countries where the vaccines are widely used[28]. To cover these pitfalls, novel vaccine strategies which target conserved viral antigens are currently being investigated[14].

We hypothesize that such vaccines can be developed by utilizing cytotoxic $CD8^+$ T cell-mediated immunity because it mainly targets viral nucleoprotein which is well conserved among a wide range of influenza virus strains[3]. Here we briefly review the current knowledge of cell-mediated immunity to influenza virus infections based on the studies using mouse models and discuss the future application of this immunological arm to the vaccines against HPAI in poultry.

Cell-mediated immunity to influenza virus infections in mouse models

1. Effector- versus central-memory T cells

Cell-mediated immunity to influenza viruses are mediated by antigen-experienced "memory" $CD8^+$ T cells[1]. Memory T cells differ from naïve T cells: i.e. they persist at a higher frequency, require lower co-stimulation for activation and are ready to respond more quickly to the re-infection. This enables animals to mount an accelerated and enhanced "recall" response to a secondary infection. Therefore, to develop vaccines which elicit cell-mediated immunity, it is essential that we understand the mechanisms of the generation, maintenance and recall of memory $CD8^+$ T cells[13].

Memory $CD8^+$ T cells are very heterogeneous in terms of phenotype, location, function, and longevity. However, the current paradigm simply classifies memory T cells into two major subsets, "effector" and "central" memory T cells[25]. Effector-memory T cells express low levels of CD62L and CCR7 and are preferentially distributed in non-lymphoid peripheral tissues. In contrast, central-memory T cells express high levels of CD62L and CCR7 and are preferentially distributed in secondary lymphoid organs.

Following recovery from an influenza infection, a large part of memory $CD8^+$ T cells are distributed in the lung tissues, including the lung airways, lung parenchyma, and pleural cavity[8,9]. Lung airway memory $CD8^+$ T cells have exclusively effector-memory phenotype. The lung airway memory $CD8^+$ T cells lack constitutive cytolytic activity and do not proliferate in situ in response to antigen. However, these functions recover rapidly when the cells are removed from the airway, suggesting that lung airway environments (probably surfactants) primarily suppress the function of memory T cells.

Memory $CD8^+$ T cells are also distributed in the secondary lymphoid organs, including the spleen and mediastinal lymph nodes[22,23]. These cells comprise a mixture of both central memory and effector memory T cells. However, this phenotypic composition dramatically changes over time: i.e. whereas effector-memory phenotype is predominant at 1 month post-infection, central-memory phenotype becomes predominant at 1 year post-infection.

2. The contributions of different memory $CD8^+$ T cell subsets to the recall response

The different locations of effector- and central-memory $CD8^+$ T cells suggest that these memory subsets contribute differently to the recall response. One hypothesis is that effector-memory T cells in the lung mediate an early response at the sites of infection, whereas central-memory T cells in the secondary lymphoid organs mediate a late proliferative response. Recently, Woodland has proposed an attractive model of the recall responses to influenza virus infections based on his seminal studies[6]. His model divides the recall response into three temporally distinct phases (Fig. 1). The first phase involves effector-memory T cells residing in the lung airways, which are the first T cells to encounter the virus (Fig. 1, line 1). The second phase involves effector-memory T cells in the circulation, which are not proliferating but are directly recruited to the lung airways (Fig. 1, line 2). The third phase involves effector- and central-memory T cells residing in the secondary lymphoid organs, which proliferate in response to antigen and are recruited to the lung airways as fully matured effector T cells (Fig. 1, line 3).

The mechanisms by which memory $CD8^+$ T cells in the lung airways contribute to the virus clearance are still unclear because these cells lack constitutive cytolytic activity and do not proliferate in situ[8,9]. Interestingly, the numbers of memory $CD8^+$ T cells in the lung airways decline over the first 6 months post-infection, whereas the numbers of memory $CD8^+$ T cells in the secondary lymphoid organs are relatively stable over time[8,9]. However, the overall efficacy of the protective cell-mediated immunity is well correlated with this progressive decline of memory $CD8^+$ T cells in the lung airways[8,9,18]. This highlights that the peripheral memory subset is critical in the cell-mediated immunity to influenza virus infections.

It is also not clear how different memory $CD8^+$ T cell

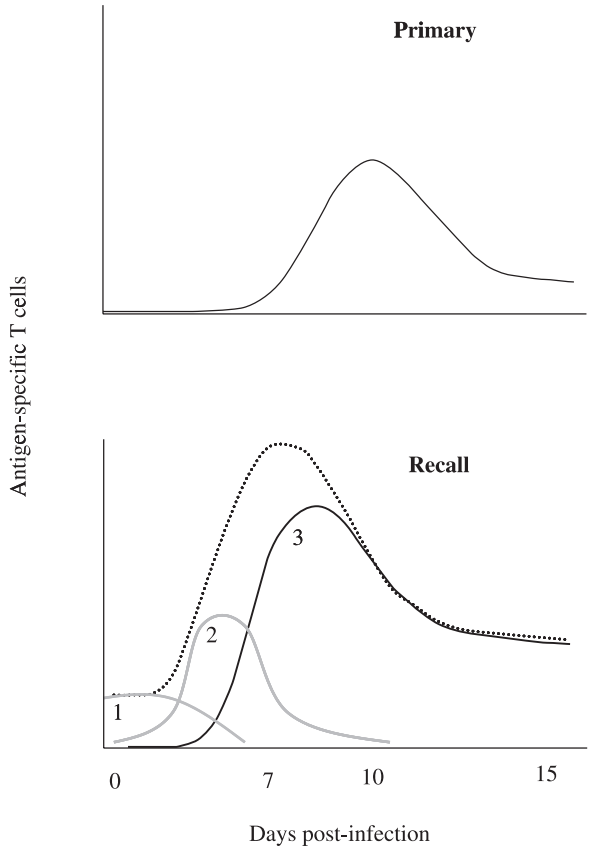

Fig. 1. Kinetics and composition of primary and recall CD8⁺ T cell responses to influenza virus infections in the lung airways

The primary response is mediated exclusively by virus-specific CD8⁺ T cells which proliferate in response to antigens in secondary lymphoid organs (top panel). In contrast, the recall response is mediated by three virus-specific memory CD8⁺ T cell subsets (see text; bottom panel). Non-proliferating memory T cells are indicated by the gray lines, whereas proliferating memory T cells are indicated by the black line. The total virus-specific memory CD8⁺ T cell response is indicated by the dotted line.

subsets in the secondary lymphoid organs contribute to the recall response. Dual adoptive transfer studies have shown that, surprisingly, the relative contributions of effector- and central-memory T cells change over time[22,23]. Whereas effector-memory CD8⁺ T cells have better recall efficacy at 1 month post-infection, central-memory CD8⁺ T cells have better recall efficacy at 12 months post-infection. Thus, it should be emphasized here that there is no direct correlation between effector-/central-memory phenotype and the recall efficacy[33]. Hikono and his colleagues have recently proposed that activation markers, such as CD27 and CD43, are superior to effector-/central-memory phenotype in predicting the recall efficacy of memory CD8⁺ T cell subsets[7]. This hypothesis may make a decisive contribution to identify a target memory CD8⁺ T cell subset which future vaccines against influenza viruses need to elicit in the secondary lymphoid organs.

Cell-mediated immunity to highly pathogenic avian influenza

There are only a few studies in which the protective potential of cell-mediated immunity to HPAI is evaluated in poultry. In the outbreak of highly pathogenic H5N1 viruses in Hong Kong in 1997, these H5N1 viruses did not cause disease signs in most of the chickens in poultry markets where H9N2 viruses concurrently circulated[26]. This observation suggested the presence of the cross-reactive cell-mediated immunity between these two serologically different subtypes. Further studies have shown that, although it allows virus shedding, the immunization of chickens with H9N2 virus indeed confers CD8⁺ T cell-mediated immunity to lethal H5N1 virus challenge[12,27]. Interestingly, the decrease of the overall efficacy of this cell-mediated immunity to HPAI well correlates with the decrease in the numbers of CD8⁺ T cells expressing interferon gamma in the lung. This highlights that the peripheral memory subset is critical in the cell-mediated immunity to HPAI in poultry[27].

It should be noted here that the biological characters of HPAI in poultry are different from influenza in mouse models. For example, HPAI viruses replicate in multiple organs and cause highly lethal infections in chickens, while mouse-adopted influenza viruses replicate only in the lung in mice[30,32]. Therefore, the protective potential of the cell-mediated immunity to HPAI needs to be further evaluated in poultry. We are currently developing the immunological methods, such as ELISPOT and intracellular interferon gamma staining, to analyze HPAI-specific memory CD8⁺ T cells in poultry.

Vaccine methods to elicit cell-mediated immunity

We now hypothesize that a successful vaccine which can elicit cell-mediated immunity to HPAI needs to establish memory CD8⁺ T cells in the peripheral non-lymphoid organs, such as lung and intestines, as well as in the secondary lymphoid organs. However, we do not know how different vaccine methods affect the generation, maintenance and recall of memory CD8⁺ T cells. Hikono and his colleagues have recently shown in the mouse model that the subcutaneous vaccination of an antigen peptide with complete Freund's adjuvant elicits large numbers of memory CD8⁺ T cells in the spleen but few in the lung[7]. In addition, this vaccine method elicits poor

memory CD8+ T cell subsets in terms of the recall efficacy[7]. This study highlights that vaccine methods, including type, route of administration and adjuvant, critically affect the vaccine efficacy through the generation and function of memory CD8+ T cell subsets.

We still do not know which vaccine methods can efficiently elicit cell-mediated immunity to influenza virus infections. In theory, live attenuated influenza virus vaccines, such as a cold-adapted influenza virus vaccine in humans, can mimic the virus infections and are likely most efficient to elicit cell-mediated immunity[5,21]. However, such vaccines are not options for HPAI in poultry because of the possibility of the accidental generation of highly pathogenic reassortants. Live virus vector vaccines based on the avian respiratory viruses, such as Newcastle disease virus, and DNA vaccines are potent candidates to elicit cell-mediated immunity in poultry[4,15,16,20,24,31]. We are currently investigating the applications of these vaccine methods to HPAI in poultry.

Conclusions

The development of the vaccines which elicit cell-mediated immunity is one of the targets toward the efficient control of HPAI in poultry. CD8+ T cell-mediated immunity would not promote antigenic drifts in HA of target HPAI viruses and also is likely to be effective against a wide range of HA-antigenic variants. Vaccines of this type can be used as a supplement or an alternative for the conventional HA-based inactivated vaccines. The rational development of such novel vaccines needs our further understanding of the generation, maintenance and recall of memory CD8+ T cells in poultry.

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